REMARKS/ARGUMENTS

Claims 1-32 are pending in this application. Claims 1-32 stand rejected for the reasons indicated in the Office Action. In response, the specification, and claims 1, 8, 9, 16, 17, 24, 25 and 32 have been amended. The specification and claims 4, 12, 15, 20, 28 and 31 have been amended to correct a minor typographical error. No new matter is added by this amendment. Entry of these amendments is hereby requested.

With Respect to the Rejections under 35 U.S.C. § 112, Second Paragraph, Page 2 of the Office Action:

Claims 8, 16, 24 and 32 stand rejected under 35 U.S.C. § 112, second paragraph for the reason indicated on page 2 of the Office Action. In response, the specification and claims 8, 16, 24 and 32 have been amended to correct a minor spelling error, and claims 8, 16, 24 and 32 have been amended to provide the proper antecedent basis for one term in these claims. The specification and claims 4, 12, 15, 20, 28 and 31 have been amended to correct a minor typographical error. Therefore, the Applicant requests that the rejections under 35 U.S.C. § 112, second paragraph be withdrawn.

With Respect to the Rejections under 35 U.S.C. §102(e), Pages 2-4 of the Outstanding Office Action:

Claims 1-7, 9-15, 17-23 and 25-31 stand rejected under 35 U.S.C. § 102(e) as being anticipated by United States Patent 6,232,107 to Bryan et al. for the reasons discussed on pages 2-4 of the Office Action. The Applicant notes that the Patent and Trademark Office indicates alternately that the rejections are being made under 35 U.S.C. § 102(b) rather than 35 U.S.C. § 102(e) on the second to last line on page 2 of the Office Action. The Applicant believes that this is a typographical error and is treating the rejections as being made under 35 U.S.C. § 102(e). If this is not correct, the Applicant requests clarification in the next Office Action.

United States Patent 6,232,107 to Bryan et al. (the '107 Patent) appears to be directed to isolated and purified nucleic acids, and the proteins they encode, that have a variety

of uses. Part of the disclosure of the '107 Patent is directed to reciting uses for the claimed nucleic acids, and the proteins they encode. There does not, however, appear to be any place in the '107 Patent that discloses new methods of use, *per se*, only recitation of methods that were in the prior art at the time the application was filed that became the '107 and that could be used with the claimed subject matter of the '107 Patent.

The Applicant has carefully examined the passages of the '107 Patent cited as the bases for rejection of the claimed subject matter of claims 1, 9, 17 and 25 of the present invention. The passages are as follows:

> The nucleic acids provide an opportunity to produce luciferases and GFPs, which have advantageous application in all areas in which luciferase/luciferins and GFPs have application. The nucleic acids can be used to obtain and produce GFPs and GFPs from other, particularly Renilla species using the probes described herein that correspond to conserved regions (see, e.g., FIG. 3). These GFPs have advantageous application in all areas in which GFPs and/or luciferase/luciferins have application. For example, The GFP's provide a means to amplify the output signal of bioluminescence generating systems. Renilla GFP has a single excitation absorbance peak in blue light (and around 498 nm) and a predominantly single emission peak around 510 nm (with a small shoulder near 540). This spectra provides a means for it to absorb blue light and efficiently convert it to green light. This results in an amplification of the output. When used in conjunction with a bioluminescence generating system that yields blue light, such as Aequorea or Renilla or Vargula (Cypridina), the output signal for any application, including diagnostic applications, is amplified. In addition, this green light can serve as an energy donor in fluorescence-based assays, such as fluorescence polarization assays, FET [fluorescent energy transfer] assays, FRET [fluorescent resonance energy transfer] assays and HTRF [homogeneous time-resolved fluorescence] assays. Particular assays, herein referred to as BRET [bioluminescence resonance energy transfer assays in which energy is transferred from a bioluminescence reaction of a luciferase to a fluorescent protein], are provided.
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> Non-radioactive energy transfer reactions, such as FET or FRET, FP and HTRF assays, are homogeneous luminescence

assays based on energy transfer that are carried out between a donor luminescent label and an acceptor label [see, e.g., Cardullo et al. (1988) Proc. Natl. Acad. Sci. U.S.A. 85:8790-8794; Peerce et al. (1986) Proc. Natl. Acad. Sci. U.S.A. 83:8092-8096; U.S. Pat. Nos. 4,777,128; 5,162,508; 4,927,923; 5,279,943; and International PCT Application No. WO 92/01225]. Non-radioactive energy transfer reactions using GFPs have been developed [see, International PCT application Nos. WO 98/02571 and WO 97/28261].

Mutagenesis of the GFPs is contemplated herein, particularly mutagenesis that results in modified GFPs that have red-shifted excitation and emission spectra. The resulting systems have higher output compared to the unmutagenized forms. These GFPs may be selected by random mutagenesis and selection for GFPs with altered spectra or by selected mutagenesis of the chromophore region of the GFP.

Recombinant host cells containing heterologous nucleic acid encoding a Renilla or Ptilosarcus GFP are also provided. In certain embodiments, the recombinant cells that contain the heterologous DNA encoding the Renilla or Ptilosarcus GFP are produced by transfection with DNA encoding a Renilla or Ptilosarcus GFP or by introduction of RNA transcripts of DNA encoding a Renilla or Ptilosarcus protein. The DNA may be introduced as a linear DNA fragment or may be included in an expression vector for stable or transient expression of the encoding DNA.

In certain embodiments, the cells contain DNA or RNA encoding a Renilla mulleri GFP or a Ptilosarcus GFP (particularly from a species other than P. gurneyi) also express the recombinant Renilla mulleri GFP or Ptilosarcus polypeptide. It is preferred that the cells are selected to express functional GFPs that retain the ability to fluorescence and that are not toxic to the host cell. In some embodiments, cells may also include heterologous nucleic acid encoding a component of a bioluminescence-generating system, preferably a photoprotein or luciferase. In preferred embodiments, the nucleic acid encoding the bioluminescence-generating system component is isolated from the species Aequorea, Vargula, Pleuromamma, Ptilosarcus or Renilla. In more preferred embodiments, the bioluminescence-generating system component is a Renilla mulleri luciferase including the amino acid sequence set forth in SEQ ID No. 18 or the Pleuromamma luciferase set forth in SEQ ID No. 28, or the Gaussia luciferase set forth in SEQ ID No. 19.

The GFPs provided herein may be used in combination with any suitable bioluminescence generating system, but is preferably used in combination with a Renilla or Aequorea, Pleuromamma or Gaussia luciferase.

Purified Renilla GFPs, particularly Renilla mulleri GFP, and purified Renilla reniformis GFP peptides are provided. Presently preferred Renilla GFP for use in the compositions herein is Renilla mulleri GFP including the sequence of amino acids set forth in SEQ ID No. 16. Presently preferred Renilla reniformis GFP peptides are those containing the GFP peptides selected from the amino acid sequences set forth in SEQ ID Nos 19-23.

The Renilla GFP, GFP peptides and luciferase can be isolated from natural sources or isolated from a prokaryotic or eukaryotic cell transfected with nucleic acid that encodes the Renilla GFP and/or luciferase protein.

Fusions of the nucleic acid, particularly DNA, encoding Renilla or Ptilosarcus GFP with DNA encoding a luciferase are also provided herein.

The cells that express functional luciferase and/or GFP, which may be used alone or in conjunction with a bioluminescence-generating system, in cell-based assays and screening methods, such as those described herein.

Presently preferred host cells for expressing GFP and luciferase are bacteria, yeasts, fungi, plant cells, insect cells and animal cells. [column 10, line 8, through column 11, line 42]

Methods for diagnosis and visualization of tissues in vivo or in situ using compositions containing a Renilla mulleri GFP and/or a Renilla mulleri luciferase or others of the luciferases and/or GFPs provided herein are provided. For example, the Renilla mulleri GFP protein can be used in conjunction with diagnostic systems that rely on bioluminescence for visualizing tissues in situ. The systems are particularly useful for visualizing and detecting neoplastic tissue and specialty tissue, such as during non-invasive and invasive procedures. The systems include compositions containing conjugates that include a tissue specific, particularly a tumor-specific, targeting agent linked to a targeted agent, a Renilla mulleri GFP, a luciferase or luciferin. The systems also include a second composition that contains the remaining components of a bioluminescence generating reaction and/or the Renilla mulleri GFP. In some embodiments, all components, except for activators, which are provided in situ or

> are present in the body or tissue, are included in a single composition. [column 12, line 66, through column 13, line 17]
>
> FIG. 11 illustrates the underlying principle of Bioluminescent Resonance Energy Transfer (BRET) and its use as sensor: A) in isolation, a luciferase, preferably an anthozoan luciferase, emits blue light from the coelenterazine-derived chromophore; B) in isolation, a GFP, preferably an anthozoan GFP that binds to the luciferase, that is excited with blue-green light emits green light from its integral peptide-based flurophore; C) when the luciferase and GFP associate as a complex in vivo or in vitro, the luciferase non-radiatively transfers its reaction energy to the GFP flurophore, which then emits the green light; D) any molecular interaction that disrupts the luciferase-GFP complex can be quantitatively monitored by observing the spectral shift from green to blue light.
>
> The nucleic acids, and the constructs and plasmids herein, permit preparation of a variety of configurations of fusion proteins that include an anthozoan GFP, such as Renilla, with its cognate anthozoan luciferase. The nucleic acid encoding the GFP can be fused adjacent to the nucleic acid encoding the luciferase or separated therefrom by insertion of nucleic acid encoding, for example, a ligand-binding domain of a protein of interest. The GFP and luciferase will be bound. Upon interaction of the ligand-binding domain with the test compound or other moiety, the interaction of the GFP and luciferase will be altered thereby changing the emission signal of the complex. If necessary the GFP and luciferase can be modified to fine tune the interaction to make it more sensitive to conformational changes or to temperature or other parameters. [column 87, lines 35-63]

All of these cited passages appear to disclose prior art methods at the time the application was filed that became the '107 Patent, none of which is the invention claimed in the present application. Fig. 11 and column 87, lines 35-63 describing Fig. 11, for example, appear to disclose a method ("BRET") where a GFP is used to amplify a signal from a luciferase, and in which a ligand binding to the luciferase disrupts the amplification ALREADY TAKING PLACE. This is clearly depicted in Fig. 11 and is clearly disclosed in the cited pages at column 87, lines 45-48:

> ...D) any molecular interaction that disrupts the luciferase-GFP complex can be quantitatively monitored by

observing the spectral shift from green to blue light.

Independent claims 1, 9, 17 and 25 have been amended to more clearly distinguish the presently claimed invention over the cited prior art methods disclosed in the '107 Patent by adding the following limitation:

> where the lack of fluorescence of the acceptor fluorophore resulting from the lack of luminescence resonance energy transfer from the donor luciferase indicates that the first protein has not interacted with the second protein.

None of the cited passages in the '107 Patent appear to teach or to suggest a method as claimed in the claims 1, 9, 17 and 25 of the present invention, where the interaction between two molecules is determined by (emphasis added):

> a) providing the first [molecule] complexed to a donor luciferase and the second [molecule] complexed to an acceptor fluorophore within the cell;
>
> b) placing the complexed first [molecule] and the complexed second [molecule] in proximity to each other within the cell; and
>
> c) detecting any fluorescence from the acceptor fluorophore;
>
> where the donor luciferase is capable of luminescence resonance energy transfer to the acceptor fluorophore when the first [molecule] is in proximity to the second [molecule]; and
>
> where fluorescence of the acceptor fluorophore resulting from luminescence resonance energy transfer from the donor luciferase indicates that the first [molecule] has interacted with the second [molecule]; and
>
> **where the lack of fluorescence of the acceptor fluorophore resulting from the lack of luminescence resonance energy transfer from the donor luciferase indicates that the first [molecule] has not interacted with the second [molecule] .**

Claims 2-7, 10-15, 18-23 and 26-31 depend on claims 1, 9, 17 and 25. As indicated above, amended claims 1, 9, 17 and 25 are believed to be in condition for allowance. Therefore, the Applicant requests that the rejection of claims 2-7, 10-15, 18-23 and 26-31 be withdrawn.

With Respect to the Rejections under 35 U.S.C. §103, Pages 7 through 10 of the Outstanding Office Action:

Claims 8, 16, 24 and 32 stand rejected under 35 U.S.C. §103(a) as being obvious over United States Patent 6,232,107 to Bryan et al. in view of United States Patent 6,872,871 to Brisson et al. for the reasons indicated on page 5 of the Office Action. Claims 8, 16, 24 and 32 depend on amended claims 1, 9, 17 and 25, respectively. As indicated above, amended claims 1, 9, 17 and 25 are believed to be in condition for allowance. Therefore, the Applicant requests that the rejection of claims 8, 16, 24 and 32 be withdrawn.

CONCLUSION

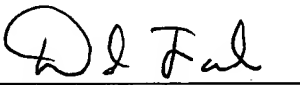
The Applicant believes that all pending claims, claims 1-32 are now in condition for allowance and a Notice of Allowance is requested. If, however, there remain any issues which can be addressed by telephone, the Examiner is encouraged to contact the undersigned.

Please deduct all fees associated with this communication, including all extension fees, from Deposit Account No. 19-2090.

Respectfully submitted,

SHELDON & MAK PC

Date: August 29, 2005

By: 

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